Chemical changes of thermally sterilized broccoli puree during shelf-life: investigation of the volatile fraction by fingerprinting-kinetics

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Abstract

For the first time in literature, this study evaluates the potential of a “fingerprinting-kinetics” strategy to investigate how the volatile fraction of thermally sterilized broccoli puree is affected by shelf-life. Samples were stored at four storage temperatures (20 °C, 28 °C, 35 °C and 42 °C). The volatile fraction was analyzed using headspace GC-MS fingerprinting as a function of time and temperature (kinetics). Fingerprinting enabled selection of sulfur-containing compounds (dimethyl sulfide, carbon disulfide, dimethyl pentasulfide, dimethyl trisulfide, bis-(methylthio)-phosphine and methyl (methylthio)methyl disulfide), nitriles (heptanonitrile, 5-methyl-hexanenitrile, hexanenitrile and 5-(methylthio)-pentanenitrile), fatty acid derivatives (octanal and 2-ethyl-1-hexanol), furanic compounds (2-ethylfuran) and Strecker aldehydes (3-methylbutanal) as volatiles significantly changing during shelf-life. In general, most of the volatiles decreased as a function of shelf-life. Next, the suitability of the volatiles (selected by fingerprinting) as markers for accelerated shelf-life testing was investigated. Based on the applied kinetic modelling, 3-methylbutanal was identified as a potential marker.

Keywords

Broccoli puree; shelf-life; chemical reactions; thermal sterilization; headspace GC-MS fingerprinting; kinetic modelling; accelerated shelf-life testing.

1. INTRODUCTION

Broccoli (*Cruciferae*) is considered to be a healthy vegetable. It contains not only a high concentration of antioxidants (such as vitamin C), but also a large quantity of glucosinolates (glucose and sulfur–containing organic compounds). Glucosinolates can be hydrolyzed both enzymatically and non-enzymatically to form different degradation products, e.g. isothiocyanates, sulforaphane and indol-3-carbinol. Although glucosinolates by themselves are biologically inactive, their listed hydrolysis products act as allelochemicals: compounds which have potential health-benefits such as antibacterial and prevention of chronic diseases (Holst and Williamson, 2004; Van Eylen et al., 2009; Aires, Carvalho & Rosa, 2012). Besides their desired effect on health, the degradation reactions also give rise to the formation of a wide range of (un)desirable odor-active compounds which affect sensory perception and consumer acceptance of broccoli. Some of the flavouring compounds are sulfides, isothiocyanates, aliphatic aldehydes and alcohols (CHIN and Lindsay, 1993; Dan, Nagata & Yamashita, 1998; Jacobsson, Nielsen & Sjoholm, 2004). Due to its high water activity (0.97 - 0.99) and low acidity (pH > 4.6), broccoli is a highly perishable commodity with a relatively short shelf-life (Raju, Chauhan & Bawa, 2011). Thermal processing is often used to increase the stability and extend shelf-life of broccoli-based foods (May, 2001; Barrett and Lloyd, 2012). Unfortunately, the intensive thermal load has a negative impact on colour, flavour, texture and vitamin content (Arnoldi, 2001; Oliveira, 2004; Awuah, Ramaswamy & Economides, 2007; Antonia Murcia, Jimenez & Martinez-Tome, 2009). In most studies investigating the process-induced impact on broccoli, the quality was studied solely immediately after processing. However, the best before date of sterilized foods is limited due to chemical changes taking place during shelf-life (van Boekel et al., 2010). Therefore, quality evaluation during shelf-life should also be taken into account to determine product acceptability (van Boekel et al., 2010).

The goal of the present work was to investigate shelf-life changes of thermally sterilized broccoli puree. Sterilized purees were stored at four storage temperatures (20 °C, 28 °C, 35 °C and 42 °C). The volatile fraction of broccoli samples was analyzed using a headspace solid-phase microextraction GC-MS (HS-SPME-GC-MS) fingerprinting as a function of time and temperature (kinetics). The obtained GC-MS were analyzed at two levels. At the first level, the potential of fingerprinting in combination with multivariate data analysis (MVDA) was used for selecting volatiles and reactions significantly changing during shelf-life. At the second level, with the aid of kinetic modeling, the suitability of the volatiles (selected by fingerprinting) as markers for accelerated shelf-life testing (ASLT) was tested. In the latter case, since conducting shelf-life study at standard storage condition can be quite resource consuming, ASLT could be an interesting method to reduce the time needed to obtain shelf-life data by accelerating quality-depletion kinetics (Mizrahi, 2000; Corradini and Peleg, 2007). For this, sterilized products were stored under temperature-abuse conditions. To the best of our knowledge, no studies evaluated the potential of the "fingerprinting-kinetics" strategy for monitoring chemical reactions occurring during shelf-life of thermally sterilized broccoli puree.

2. MATERIALS AND METHODS

2.1. Sample preparation

A single batch of freshly harvested broccoli (*Brassica oleracea* cv. Southern comet) was purchased at a local market. The broccoli was carefully washed and cut into small florets. The florets were packed into low-density polyethylene bags. To prevent enzymatic reactions during processing, storage and thawing, the packaged broccoli was blanched at 95 °C for 8 min in a water bath (Haake W15 DC-10, Clausthal-Zellerfeld, Germany). The blanching conditions were validated using a qualitative and quantitative peroxidase test (Kebede et al., 2013). After

95 blanching, the plastic bags were immediately cooled in ice water for 10 min, frozen in liquid
96 nitrogen and stored in a freezer at -40 °C until processing. Prior to processing, the samples were
97 thawed overnight at 4 °C. In order to prepare the puree, deionized water was added to the
98 blanched broccoli, blended for 1 min using a Buchi mixer (B-400, BUCHI, Switzerland) and
99 further homogenized by high pressure homogenization (at 1000 bar while temperature was
100 maintained < 4 °C) (Panda 2K, Gea Niro Soavi, Mechelen, Belgium).

101 **2.2. Thermal processing**

102 The thermal treatment was carried out in a static steriflow pilot retort (Barriquand, Paris,
103 France). An industrially-relevant sterilization value of $F_{121.1^0C}^{10^0C}$ (F_0) = 5 min was selected. Glass
104 jars (100 ml volume, 95 mm height and 45 mm diameter) were filled with 85 ± 0.5 g of broccoli
105 puree and closed with metal lids. Temperature profiles in the retort and at the coldest point of the
106 product were recorded using type T thermocouples (Ellab, Hillerød, Denmark). The data logging
107 device provided real-time information of the whole process. For the graphical representation of
108 exemplary time-temperature profiles of the product and environment during treatment, the reader
109 is referred to Kebede et al. (2013). Following completion of the treatments, samples were
110 transferred to ice water to further cool the product down.

111 **2.3. Storage**

112 Sterilized glass jars were stored in incubators, protected from light, at 20 °C and 28 °C up to 44
113 weeks, at 35 °C up to 26 weeks and at 42 °C up to 18 weeks. At fixed points in time (11 points
114 per temperature), glass jars were sampled from the incubators. The vegetable puree was
115 aseptically (next to a Bunsen burner) transferred to small-volume (10 ml) polyethylene
116 terephthalate tubes with a polyethylene cap. One gram of sample was taken for microbial

117 analysis. Thereafter, the tubes were frozen in liquid nitrogen, wrapped with aluminium foil and
118 stored in freezer at -40 °C until GC-MS analysis.

119 **2.4. Microbial analysis**

120 Microbial analysis was performed to verify growth of mesophilic (aerobic) and thermophilic
121 (aerobic and anaerobic) microorganisms. Plate count agar was prepared for aerobic (mesophilic
122 and thermophilic) bacteria, whereas the presence of anaerobic thermophiles were analyzed using
123 reinforced clostridial agar. For all investigated shelf-life time and temperature conditions, the
124 microbial growth was below detection limit (results not shown).

125 **2.5. HS-SPME-GC-MS analysis**

126 Samples were thawed overnight in the cooling room (4 °C). 2.5 g thawed sample and 2.5 ml
127 saturated NaCl solution were mixed into a 10 ml amber glass vial (10 ml, VWR International,
128 Radnor, PA, USA). The vials were tightly closed using screw-caps with silicon septum seal
129 (GRACE, Columbia, MD, USA), mixed and transferred to the cooling tray of the auto-sampler
130 which was maintained at 10 °C. Headspace fingerprinting was conducted on a gas
131 chromatography (GC) system (7890N, Keysight Technologies, Diegem, Belgium) coupled to a
132 mass selective detector (MSD) (5977N, Keysight Technologies, Diegem, Belgium) and equipped
133 with a combipal autosampler (CTC analytics, Zwingen, Switzerland). Targeting detection of a
134 wide range of volatiles in a particular food extract, a HS-SPME-GC-MS method of analysis was
135 optimized beforehand (Kebede et al., 2014b). In the selected method, the samples were incubated
136 at 40 °C during 20 minutes under agitation at 500 rpm. Next, extraction of the volatiles was
137 performed using a HS-SPME fiber coated with 30/50 µm
138 divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) (StableFlex, Supelco,
139 Bellefonte, PA, USA) at 40 °C during 10 min. The SPME fiber was inserted into the heated (230

°C) GC-injection port for 2 min to desorb the volatile compounds. Prior to extraction, the fibers were conditioned and regenerated according to the manufacturer's guidelines in the conditioning station of the auto-sampler. Injection of the samples to the GC-column was performed in split (1/5) mode. Chromatographic separation was carried out on a HP-5MS capillary column (30 m ×0.25 mm i.d., 0.25 μm film thickness, Agilent Technologies J&W, Santa Clara, CA, USA) coated with 5%-phenyl-methylpolysiloxane as a stationary phase and helium as a gas phase at a constant flow of 1.2 mL/min. The GC-oven temperature was programmed from a starting temperature of 40 °C, which was retained for 2 min, to 172 °C at 4 °C/min, then ramped to 300 °C at 30°C/min and kept constant at 300 °C for 2 min before cooling back to 40 °C. The mass spectra were obtained by electron ionization (EI) mode at 70 eV with a scanning range of 35 to 400 m/z and a scanning speed of 3.8 scans per second. MS ion source and quadrupole temperatures were 230 °C and 150 °C, respectively. In this work, to minimize the phenomenon of fiber degradation, a new fiber was used for each storage temperature. Per storage temperature, during analysis, the samples were randomized as a function of storage time. Possible fiber degradation was carefully monitored by analysis of a reference sample (blanched carrot samples), every 10 injections. Per storage time and temperature condition, the analysis was repeated six times.

2.6. Data analysis

2.6.1. Data pre-processing

As commonly observed in GC-MS analysis, co-eluting compounds were present in the obtained chromatograms. Therefore, all chromatograms were analyzed with automated mass spectral deconvolution and identification system (AMDIS) (Version 2.66, 2008, National Institute of Standards and Technology, Gaithersburg, MD, USA) to extract "pure" component spectra from complex chromatograms. In addition, for proof of identity along with the mass spectral data,

AMDIS was used to build a retention index calibration file. The deconvoluted spectra were then analyzed with mass profiler professional (MPP) (Version 12.0, 2012, Keysight Technologies, Diegem, Belgium) for filtering and peak alignment. The MPP yielded a spreadsheet containing peak areas, which was used as an input for the multivariate statistical data analysis.

2.6.2. Multivariate data analysis

The multivariate data were analyzed with a multivariate data analysis (MVDA) which was carried out in Solo (Version 6.5, 2011, Eigenvector Research, Wenatchee, WA, USA). All data were mean-centered and the variables were weighed by their standard deviation to give them equal variance. In a first step, principal component analysis (PCA) was conducted as an exploratory technique to evaluate each data set and to detect potential outliers. To study the change in the volatile fractions during storage, per storage temperature, partial least squares (PLS) regression was performed, with the volatiles as *X*-variables and the storage time as *Y*-variable. For determining the complexity of the PLS model, the lowest number of latent variables (LVs) that maximally describe the change during storage was used. Score plots and correlation loading plots were combined in bi-plots by dividing each score through the maximal vector length of the original score plot matrix. Bi-plots were used to graphically investigate the change in the volatile fraction as a function of time. Next, to select volatile compounds clearly changing during storage, variable identification (VID) coefficients were calculated. These values correspond to the correlation coefficient between each original *X*-variable and predicted (by the selected PLS-model) *Y*-variable. In this work, variables with an absolute VID value higher than 0.700 were considered to be important. These variables were further identified and linked to possible reaction pathways. Identification of the compounds was performed by comparing the deconvoluted mass spectrum with the reference mass spectra from both NIST spectral library (NIST08, version 2.0, National Institute of Standards and Technology, Gaithersburg, MD, USA)

188 and WILEY mass spectral data (Wiley2010, version 9, Hoboken, New York, USA). For
189 identification, a threshold match of 90 % was taken into account. For further confirmation, visual
190 inspection of spectral matching between the detected compound and the match from the library
191 as well as comparison of the retention index were performed.

192 *2.6.3. Kinetic modelling and parameter estimation*

193 Kinetic modelling was performed based on the rate equation of a degradation reaction. For a
194 detailed discussion on the general principles of kinetic modeling, the reader is referred to the
195 work of van Boekel (2009).The general rate equation of an n^{th} order degradation reaction is
196 expressed as **Equation 1**, where v is the reaction rate, A is any property of interest, n is the
197 reaction order and k is the rate constant. Volatiles that were changing during storage could be
198 modelled best by a first-order kinetic model (n = 1)**,** where A_0 is the initial concentration at time t
199 = 0 (start of storage) and t is the storage time in weeks (**Equation 2**). The temperature
200 dependency of the reaction rate constant was evaluated using the Arrhenius equation (**Equation**
201 **3**), where E_a is the activation energy (kJ/mol), T is the storage temperature in kelvin, k_{ref} is the
202 rate constant (weeks^{-1}) at reference storage temperature (20 °C) and R is the gas constant
203 (kJ/molK).

204 $$v = \frac{\mathrm{dA}}{\mathrm{d}t} = -kA^n \quad (1)$$

205 $$A = A_0\, exp(-kt) \quad (2)$$

206 $$k = k_{ref} exp\left(\frac{Ea}{R}\left(\frac{1}{T_{ref}} - \frac{1}{T}\right)\right) \quad (3)$$

207 Model evaluation was performed by examining R^2_{adjusted} (**Equation 4**) and by visual inspection of
208 the parity plot (estimated values versus measured values) and the scatter plot (residuals versus
209 estimated values).

210 $$R^2_{adjusted} = 1 - \frac{\left[(\mathrm{DF_{tot}} - 1)\left(1 - \frac{\mathrm{SS_{model}}}{\mathrm{SS_{total}}}\right)\right]}{\mathrm{DF_{error}}} \quad (4)$$

211 In **Equation 4**, DF_{tot} and DF_{error} are degree of freedom of total and error, respectively and SS is
212 the sum of squares. One-step regression analysis was performed by inserting **Equation 3** in
213 **Equation 2**, using SAS® software (version 9.3, Cary, USA).

214 ## 3. RESULTS AND DISCUSSION

215 As explained in the introduction, in the present work, chemical reactions occurring during shelf-
216 life of thermally sterilized broccoli puree were investigated using "fingerprinting-kinetics"
217 approach. The sterilized samples were stored at four storage temperatures (20 °C, 28 °C, 35 °C
218 and 42 °C). The volatile fraction of broccoli samples was analyzed using a headspace solid-phase
219 microextraction GC-MS (HS-SPME-GC-MS) fingerprinting as a function of storage time and
220 temperature (kinetics).
221 The followed HS-SPME–GC–MS fingerprinting procedures and results will be discussed,
222 starting from qualitative investigation of the time-related chemical changes during shelf-life to
223 identification of important shelf-life volatiles and linkage to reaction pathways. Finally, the
224 evolution of specific selected volatile compounds during shelf-life is modelled. By evaluating the
225 estimated kinetic parameters, the suitability of these volatiles as markers for accelerated shelf-
226 life testing (ASLT) is investigated.

227 ### 3.1. Headspace GC-MS fingerprinting

228 *3.1.1. Qualitative investigation of the time-related chemical changes during shelf-life*
229 **Fig. 1** depicts an exemplary GC-MS total ion chromatogram of the headspace of thermally
230 sterilized broccoli puree at the start of storage (day 0). The complex GC–MS data files were
231 analyzed with a sequence of data preprocessing techniques (i.e., AMDIS and MPP). The MPP

obtained a spreadsheet containing peak areas, which was used as an input for the next statistical analysis (MVDA).

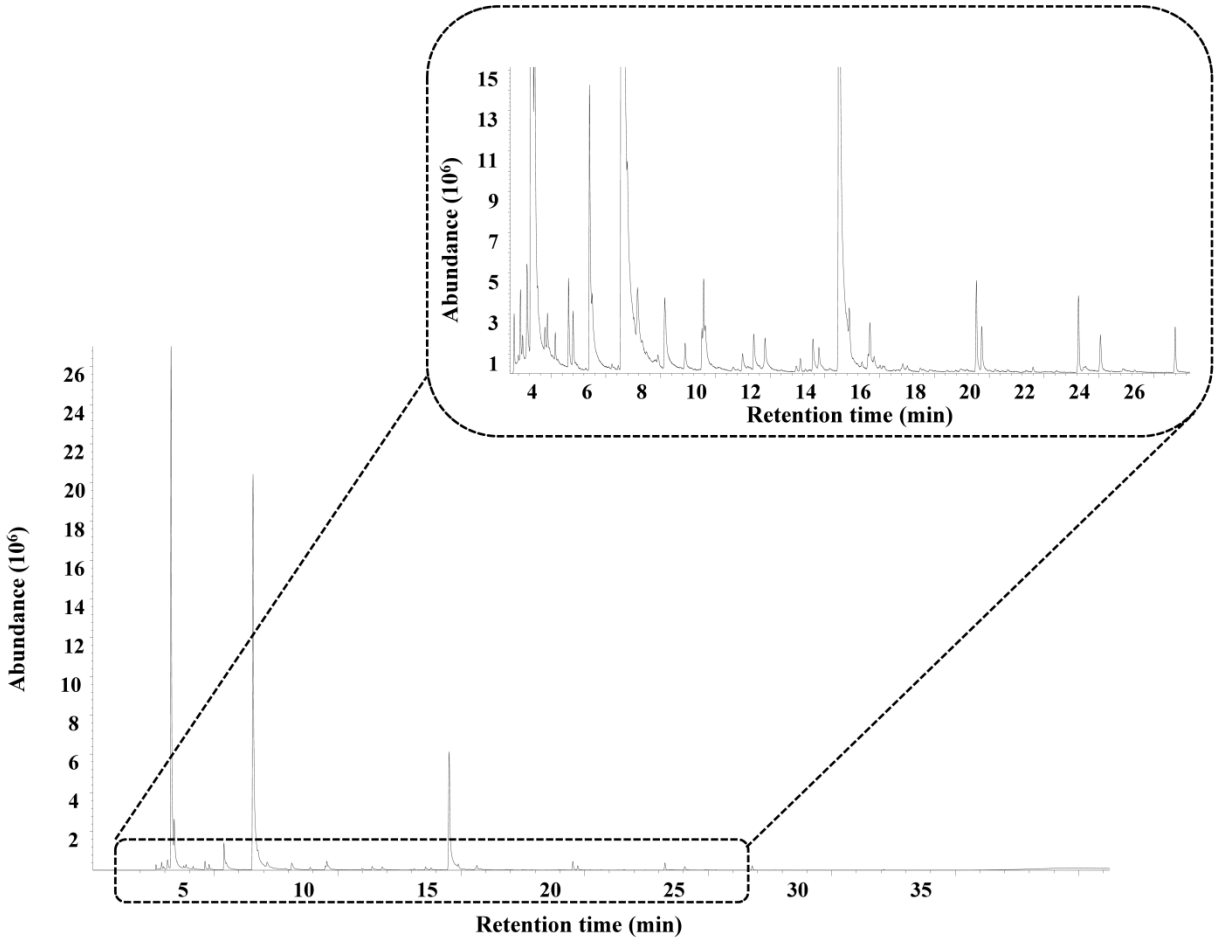
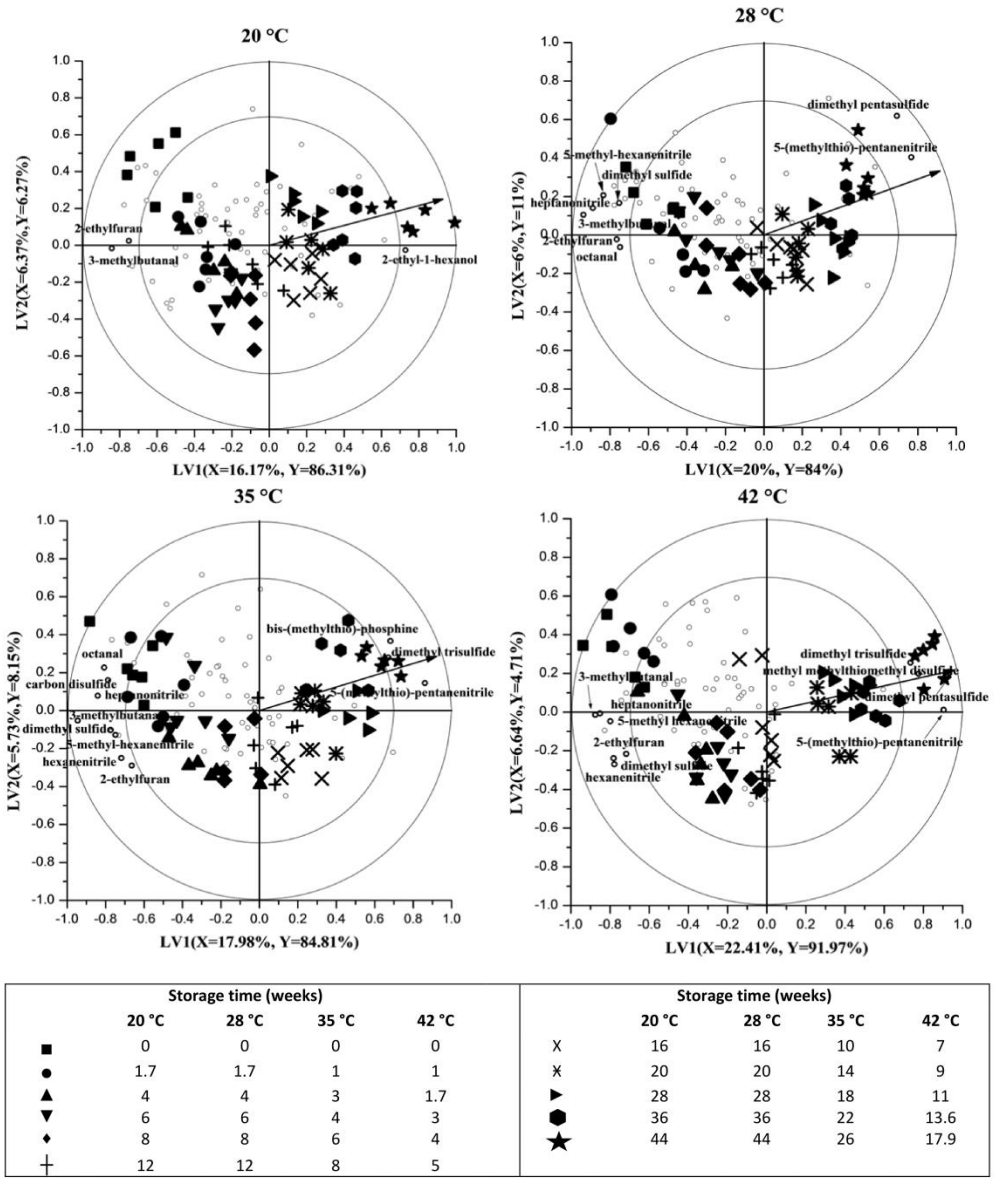


Fig. 1. Total ion chromatogram of the headspace of thermally sterilized broccoli puree at the start of storage (day 0), obtained by headspace solid-phase microextraction GC-MS (HS-SPME-GC-MS) fingerprinting.

Per storage temperature, the complex data tables were subsequently analysed with principal component analysis (PCA) as an exploratory technique, to detect outliers and observe any trend during storage (results not shown). Next, per storage temperature, a partial least squares (PLS) regression model was built based on two latent variables (LVs), which respective bi-plots are

shown in **Fig. 2**. The LVs are a linear combination of the original *X*-variables (volatiles) for which the trend as a function of storage time (continuous *Y*-variable) is maximally explained.



Storage time (weeks)				
	20 °C	28 °C	35 °C	42 °C
■	0	0	0	0
●	1.7	1.7	1	1
▲	4	4	3	1.7
▼	6	6	4	3
♦	8	8	6	4
+	12	12	8	5

Storage time (weeks)				
	20 °C	28 °C	35 °C	42 °C
X	16	16	10	7
✳	20	20	14	9
►	28	28	18	11
⬢	36	36	22	13.6
★	44	44	26	17.9

Fig. 2. PLS-bi-plots describing the effect of storage on the volatile fraction of thermally sterilized broccoli puree (objects represented by differently shaped symbols) stored at different temperatures, i.e., 20 °C, 28 °C, 35 °C, and 42 °C. The open circles represent headspace components, of which only components selected by the VID procedure are identified and marked in bold (Table 2). The vectors represent the correlation loading for the continuous *Y*-variable (time). The percentages of the *X*- and *Y*-variances explained by each latent variable (LV1 and LV2) are indicated on the respective axes.

Per storage temperature, the first two LVs explained a considerable amount of *Y*-variance (92 %, 95 %, 93 % and 97 % for 20 °C, 28 °C, 35 °C and 42 °C, respectively), with a large portion already covered by the first LV (**Fig. 2**). A bi-plot is an interesting tool to graphically represent the change in the headspace fractions during storage. As can be seen from **Fig. 2**, the trend of storage time can be clearly observed in the bi-plots. In each bi-plot, the long extended vector, which represents the correlation loading for the *Y*-variable (time), is also a good indicator that the selected two LVs were sufficient to built an appropriate model. The bi-plots also display the relation between headspace compounds and storage time. For instance, compounds that are located in the same direction as the *Y*-vector are positively correlated with storage time indicating increase in concentration as a function of storage time, while the ones that are projected in the opposite direction are negatively correlated meaning they were observed to decrease as a function of time. Most of the components (small open circles, **Fig. 2**) are projected to the beginning of shelf-life while fewer are positioned close to the end of shelf-life. This seems to suggest that the concentration of most of the headspace compounds have decreased during storage while only few compounds appear to be formed as a function of storage. Moreover, these observations seem to be comparable among the different storage temperatures. In addition, on the bi-plots, based on the distance of a component from the center of the coordinate, its importance for displaying the changes during storage can be discussed. For instance, if a compound is projected far from the center and located between the two ellipses (inner and outer ellipses represent correlation coefficients of 70 and 100 %, respectively) on the plot, this shows that the concentration of this compound has largely changed as function of storage time.

3.1.2. Identification of important shelf-life volatiles and linkage to reaction pathways

Even though the bi-plot provides graphical representation of the changes, it is not straightforward to rank the relative component importance for the change as a function of storage

time. Therefore, to quantitatively rank volatile's importance for the change, VID coefficients were calculated using the selected PLS models. Each volatile was assigned with a value between -1 and +1, where a positive VID coefficient represents a higher concentration after storage and *vice versa*.

Table 1
Volatiles significantly changing as a function of shelf-life, per storage temperature (20 °C, 28 °C, 35 °C and 42 °C), selected based on the VID procedure, listed in increasing order of VID coefficient. Positive VID coefficients signify an increase in concentration during storage while negative coefficients denote a decrease. The retention index (RI) and possible chemical group is also listed

storage temperature	VID	identity	RI	chemical group
20 °C	-0.834	3-methylbutanal	763	aldehyde (Strecker)
	-0.737	2-ethylfuran	788	furanic compound
	0.714	2-ethyl-1-hexanol	1113	alcohol
28 °C	-0.901	3-methylbutanal	763	aldehyde (Strecker)
	-0.846	heptanonitrile	1059	nitrile
	-0.780	5-methyl-hexanenitrile	1020	nitrile
	-0.755	2-ethylfuran	788	furanic compound
	-0.746	octanal	1085	aldehyde (aliphatic)
	-0.706	dimethyl sulfide	724	sulfur compound
	0.797	dimethyl pentasulfide	1323	sulfur compound
	0.829	5-(methylthio)-pentanenitrile	1035	nitrile
35 °C	-0.940	3-methylbutanal	763	aldehyde (Strecker)
	-0.815	heptanonitrile	1058	nitrile
	-0.780	dimethyl sulfide	724	sulfur compound
	-0.760	5-methyl-hexanenitrile	1020	nitrile
	-0.756	octanal	1085	aldehyde (aliphatic)
	-0.751	hexanenitrile	946	nitrile
	-0.749	carbon disulfide	727	sulfur compound
	-0.704	2-ethylfuran	788	furanic compound
	0.709	dimethyl trisulfide	1048	sulfur compound
	0.735	bis-(methylthio)-phosphine	1323	sulfur compound
	0.873	5-(methylthio)-pentanenitrile	1304	nitrile
42 °C	-0.871	3-methylbutanal	764	aldehyde (Strecker)
	-0.846	heptanonitrile	1059	nitrile
	-0.805	dimethyl sulfide	724	sulfur compound
	-0.805	hexanenitrile	946	nitrile
	-0.799	5-methyl hexanenitrile	1020	nitrile
	-0.739	2-ethylfuran	788	furanic compound
	0.760	dimethyl trisulfide	1048	sulfur compound
	0.794	methyl (methylthio)methyl disulfide	1225	sulfur compound
	0.898	5-(methylthio)-pentanenitrile	1304	nitrile
	0.949	dimethyl pentasulfide	1323	sulfur compound

Since the objective was to determine compounds highly changing in concentration, only those with absolute value higher than 0.70 were selected to further zoom into and only those were

identified (**Table 1; Fig. 2** (bold open circles)). In samples stored at storage temperatures of 20 °C, 28 °C, 35 °C and 42 °C, respectively, 3, 8, 11 and 10 volatile compounds were selected. In agreement with the discussion above, most of the volatiles were selected with a negative VID indicating a decreasing concentration as a function of shelf-life. All selected volatile compounds can be categorized under sulfur-containing compounds, nitriles, aliphatic aldehydes, alcohols, Strecker aldehydes and furanic compounds (**Table 1**). Prior to thermal sterilization, the broccoli florets were blanched. In addition, from the microbial analysis, no microbial spoilage was detected during the investigated shelf-life. Hence, enzymatic and microbial activities were not expected to have an impact on the change in volatiles, and changes can be related to chemical reactions.

In literature, the formation of sulfur-containing compounds in broccoli is linked to the degradation of both glucosinolates and sulfur-containing amino acids. Dimethyl sulfide (together with other compounds like methanethiol) is reported as the major contributor to undesirable odor of broccoli during storage, which renders the product unmarketable (Forney, Mattheis & Austin, 1991). It was reported that high temperature prompts the production of these off-odor volatiles through increasing the velocity of the chemical reactions (Jin, Wang, Rosen & Ho, 1999; Kebede et al., 2013). In line with this hypothesis, in the present work, dimethyl sulfide (together with carbon disulfide) is increasingly detected after thermal sterilization, but their concentration decreased during shelf-life. It can be hypothesized that during storage (oxidative) breakdown of these compounds into others occurred. However, in contrast with this observation, other sulfur-containing compounds, such as dimethyl pentasulfide, dimethyl trisulfide, bis-(methylthio)-phosphine and methyl (methylthio)methyl disulfide seem to increase as a function of shelf-life.

313 Nitriles have been reported to be more readily formed during thermal treatment and essentially
314 identified as common thermally-induced degradation products of glucosinolates in broccoli
315 (MacLoad, Panesar & Gil, 1981; Jones, 2008; Hanschen, Platz, Mewis, Schreiner, Rohn & Kroh,
316 2012). In this study, in agreement with the previous suggestions, most of the nitriles, such as
317 heptanonitrile, 5-methyl-hexanenitrile and hexanenitrile, were detected at higher amounts
318 immediately after thermal processing. Nevertheless, their concentration decreased during shelf-
319 life. Possibly, these compounds were unstable during storage and further degraded and/or reacted
320 with other components.
321
322 Octanal is an aliphatic aldehyde, which is mostly linked with autoxidation and/or thermally-
323 induced oxidation of unsaturated fatty acids (Heatherbell, Wrolstad & Libbey, 1971; Kebede et
324 al., 2013; Kebede et al., 2014a; Kebede et al., 2014b). In this work, even though octanal was
325 increasingly formed due to the applied thermal sterilization, its concentration decreased during
326 shelf-life. It can be hypothesized that during storage oxidative breakdown of the aldehyde into
327 other compounds occurred. This could be the possible explanation for the increase in the amount
328 of 2-ethyl-1-hexanol (an alcohol) as a function of shelf-life (**Table 1**).
329
330 The other group of selected volatiles includes 2-ethylfuran and 3-methylbutanal. These volatiles
331 are reaction products of the Maillard reaction and Strecker degradation, respectively. Although
332 Strecker degradation is a sub-reaction category of the Maillard reaction scheme, it has been
333 described in directing the Maillard reaction towards the aromagenic pathways rather than to
334 chromogenic pathways. In other words, this reaction is of outermost importance in relation to
335 flavor formation (Yaylayan, 2003; van Boekel, 2006; Rizzi, 2008). In this work, it was observed
336 that these compounds were selected with negative VID values indicating a decrease in their
337 concentration during shelf-life.

Based on the data from the fingerprinting as a function of time and temperature, a kinetic modelling of the selected volatiles was performed to investigate their reaction kinetics at different storage temperatures. Furthermore, the suitability of these volatiles as markers for ASLT was investigated.

3.2. Investigating the reaction kinetics of volatile compounds changing during shelf-life

Firstly, an appropriate kinetic model was identified. Next, kinetic parameters, such as reaction rate constants and activation energies, were estimated using a non-linear one-step (**Equation 2 and 3**) regression analysis. Of all selected compounds, 2-ethylfuran, octanal, hexanenitrile, dimethyl trisulfide and bis-(methylthio)-phosphine showed a scattering behavior (results not shown). Probably, formation of these compounds does not obey Arrhenius kinetics and hence these compounds don't seem to be interesting ASLT markers. The rest of the compounds could be adequately modelled best by means of a first-order reaction model. The kinetic parameters and their corresponding 95 % approximate confidence interval are listed in **Table 2**.

Table 2

Estimated kinetic parameters based on a regression of combination of Equation 3 in Equation 2, (20 °C as reference temperature) describing changes of volatiles compounds in broccoli puree during shelf-life. Samples were stored at 20 °C, 28 °C, 35 °C and 42 °C. The temperature quotient (Q_{10}) in the studied temperature zone is given.

compound	k_{ref} (week^{-1})	E_a (kJ/mol)	R^2_{adj}	Q_{10}
heptanonitrile	0.034 ± 0.007	14 ± 11	0.93	1.20
5-methyl hexanenitrile	0.023 ± 0.006	16 ± 14	0.93	1.23
3-methylbutanal	0.028 ± 0.003	34 ± 5	0.98	1.56
carbon disulfide	0.060 ± 0.013	79 ± 11	0.86	2.82
5-(methylthio)-pentanenitrile	-0.008 ± 0.003	87 ± 12	0.87	3.11
dimethyl pentasulfide	-0.006 ± 0.002	107 ± 14	0.90	4.05
methyl (methylthio)methyl disulfide	-0.005 ± 0.002	112 ± 13	0.92	4.28
dimethyl sulfide	0.002 ± 0.001	115 ± 17	0.98	4.50

± 95 % approximate confidence interval

359 The temperature quotient (Q_{10}) in the studied temperature zone is calculated. As an example, the
360 changes of 3-methylbutanal, heptanonitrile and dimethyl pentasulfide are presented in **Fig. 3**. In
361 the left section of the figure, compound's peak area as a function of time in thermally treated
362 broccoli puree stored at 20 °C, 28 °C, 35 °C and 42 °C is shown. The full lines represent peak
363 area predicted by the kinetic model while the experimental data are represented by the symbols.
364 The model was evaluated using parity plot (middle section of **Fig. 3**), scatter plot (right section
365 of **Fig. 3**) and $R^2_{adjusted}$ (**Table 2**). For determining which of the modelled volatiles could be
366 potential ASLT markers, two criteria were established: (i) the reaction should be temperature-
367 dependent; (ii) there should be an observable change not only at temperature-abuse conditions
368 but also at reference/ambient storage temperature (20 °C). Heptanonitrile and 5-methyl
369 hexanenitrile are characterized by very low E_a- and Q_{10} values compared to other compounds,
370 indicating a very low temperature dependency. Hence, given the very small reaction acceleration
371 by increasing storage temperature, these volatiles seem less interesting to be considered as
372 markers for ASLT. For the rest of the compounds, as can be seen from their E_a-values,
373 increasing the storage temperature effectively increased the rate constants. Nevertheless, for
374 some of them (e.g., carbon disulphide, 5-(methylthio)-pentanenitrile, dimethyl pentasulfide,
375 methyl (methylthio)methyl disulphide and dimethyl sulphide) the formation at 20 °C proceeds
376 very slow and seems to largely increase at an elevated storage temperature, as indicated by their
377 relatively high activation energies and temperature quotient (**Table 2**). As also discussed in the
378 previous section, these compounds were selected by the VID procedure at all storage
379 temperatures but not at 20 °C, indicating the strong temperature dependency of the reaction
380 kinetics. Therefore, taking into account the second criteria established in this work, care should
381 be taken if and when considering these temperature sensitive volatiles as ASLT markers.
382 Considering both established criteria, 3-methylbutanal seems to be an interesting compound. Its
383 reaction follows Arrhenius kinetics wherein higher storage temperatures lead to the acceleration

of the rate of the reaction. In addition, its rate constant is also significant at ambient storage temperature. Therefore, this compound can be considered as a marker for ASLT of thermally sterilized broccoli puree.

4. Conclusion

This study clearly showed the power of the followed fingerprinting-kinetics approach to increase insight into chemical changes during shelf-life of sterilized broccoli puree. In a first step, fingerprinting enabled selection of sulfur-containing compounds (dimethyl sulfide, carbon disulfide, dimethyl pentasulfide, dimethyl trisulfide, bis-(methylthio)-phosphine and methyl (methylthio)methyl disulfide), nitriles (heptanonitrile, 5-methyl-hexanenitrile, hexanenitrile and 5-(methylthio)-pentanenitrile), fatty acid derivatives (octanal and 2-ethyl-1-hexanol), furanic compounds (2-ethylfuran) and Strecker aldehydes (3-methylbutanal) as volatiles that are significantly changing during shelf-life in broccoli. In general, most of the volatiles decreased as a function of shelf-life. It can be concluded that the present study clearly demonstrated that quality changes linked to the broccoli volatile fraction occurs not only during processing but also during shelf-life. In the next step, the suitability of these volatiles as markers for accelerated shelf-life testing (ASLT) was investigated. By evaluating the estimated kinetic parameters, 3-methylbutanal was selected as a potential marker for ASLT of thermally sterilized broccoli puree. In general, the applicability of a marker (selected by the fingerprinting-kinetics strategy) to be used for shelf-life estimation can be categorized into two: (i) In cases the marker determines the best before date of the food product and is important for the consumer, the kinetics can be directly used for shelf-life estimation; (ii) if the marker does not directly determine the best before date, it has a potential to be used as a witness for shelf-life changes, in case the kinetics matches with the kinetics of a compound determining the best before date.

Based on the obtained results, it is difficult to evaluate to which extent the selected marker affects overall broccoli flavor. This was not the aim of the present work, but in the future it is worthwhile to link the results of the present work to a sensory analysis.

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